Determination of Mycotoxin and Mycotoxin Metabolites using LC/MS/MS

Carrie L. Young and John R. Barr
Centers for Disease Control and Prevention, Atlanta GA 30341

Jeffrey S. Snow; Roy W. Plunkett Jr., Michael F. Martin, Timothy R. Croley
Commonwealth of Virginia, Division of Consolidated Laboratory Services, Richmond VA 23219

Introduction

Mycotoxins are the secondary by-products of fungal mold metabolism. The toxicity and potential weaponization threat of mycotoxins demands the need for sensitive, robust and rugged analytical methodologies. Current methods used by agriculture and food laboratories are often unable to detect a suite of these toxins. In addition, these methods do not address exposure to these compounds, where detection of the metabolites would be necessary for clinical diagnosis and treatment. Our laboratories have developed both a GC/MS/MS and an LC/MS/MS protocol for the determination of a select group of mycotoxins and their metabolites (Figure 1) in both environmental and clinical matrices.

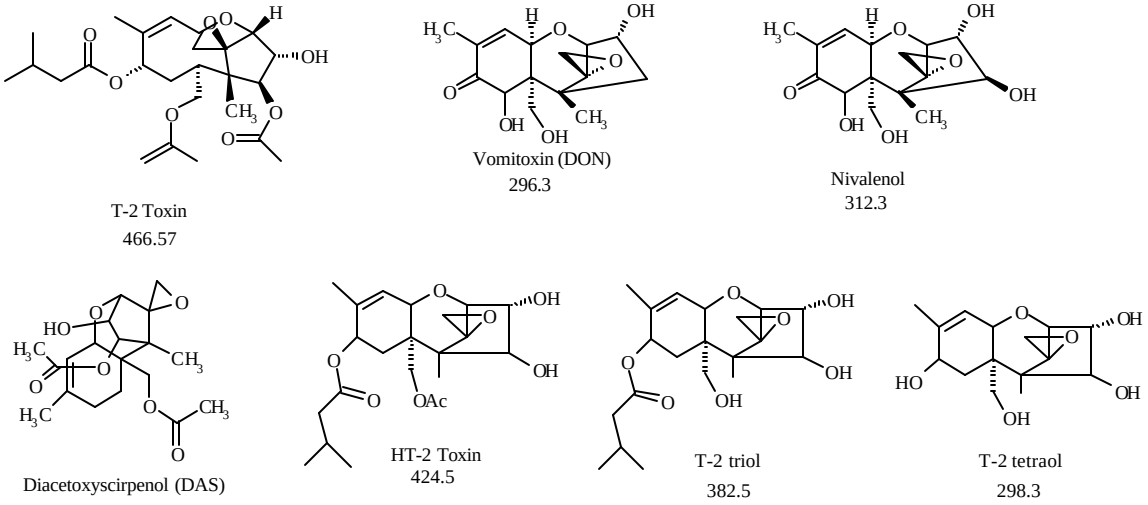

Figure 1: Selected mycotoxins and mycotoxin metabolites

Experimental

Aliquots of urine were filtered and extracted using solid-phase extraction, SPE (Varian, Harbor City, CA). Extracts were then analyzed using both GC/MS/MS and LC/MS/MS. Positive ion chemical ionization GC/MS/MS was performed on a TSQ 7000 (Finnigan MAT, San Jose, CA). HPLC (Agilent, Wilmington, DE) separation was performed on a C-18 column (Supelco, Bellefonte, PA) using at 25:75 MeOH:H_2O mobile phase. Quadrupole ion trap tandem mass spectrometry was performed on an Esquire 3000plus (Bruker Daltonics, Billerica, MA) operating in the positive ion mode.

Results

Figure 2 shows a typical HPLC/MS chromatogram for the following mycotoxins and mycotoxin metabolites: T-2, T-2 tetraol, T-2 triol, nivalenol, vomitoxin (DON), diacetoxyscirpenol (DAS). As can be seen in the figure, tetraol, nivalenol and DON have lower response than the other analytes. This is probably due to the polarity of these three compounds relative to the other four analytes. In addition, these compounds are very labile and undergo significant fragmentation to multiple product ions under any collision-induced dissociation (CID) conditions and selected ion storage (SIS) was utilized for these three compounds followed by MS/MS for the latter compounds. An alternative solution to this problem would be the use of chemical ionization GC/MS/MS utilizing a quick derivatization step. Using tetraol and triol as test compounds, similar studies were carried out. In these studies, we found comparable sensitivity with the advantage of a confirmatory product ion in the case of tetraol; however, the sensitivity for triol was somewhat better using LC/MS/MS.

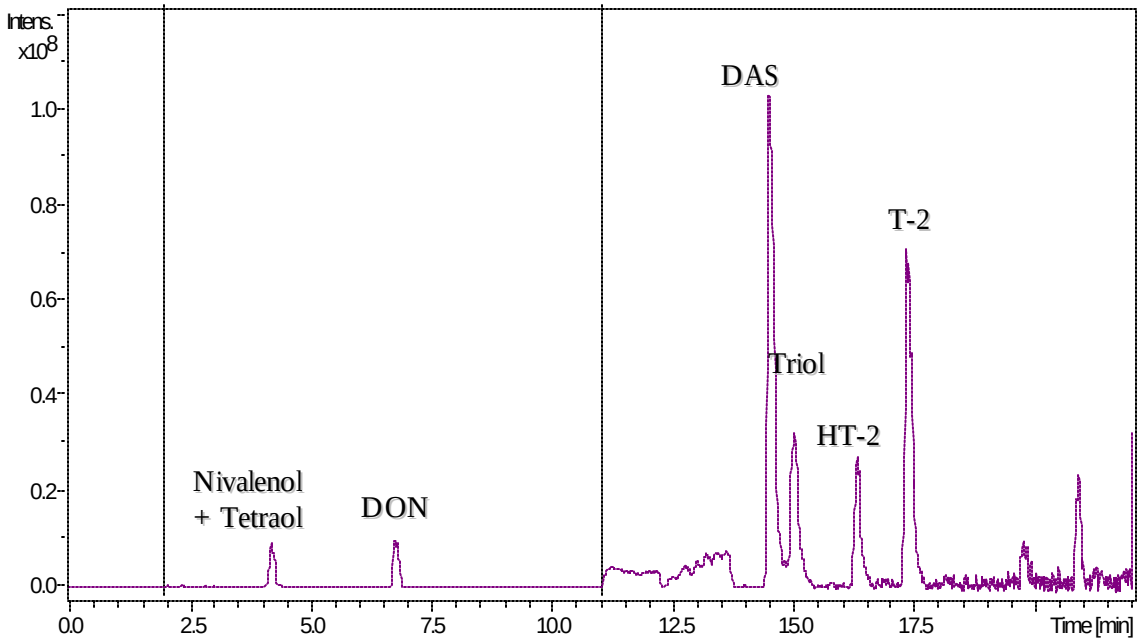

Figure 2: HPLC/MS Chromatogram of the analytes of interest.

Conclusions

We have developed two sensitive methods for the detection of mycotoxins and mycotoxin metabolites that are amenable to both biological and food matrices. These methods will provide public health laboratories with a valuable and powerful tool to respond to exposures to mycotoxins. In addition, quantitation of these compounds in clinical samples will provide healthcare with a tool for treatment of patients who have been exposed to these compounds.